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Abnormal vitamin D_3 metabolism in patients with primary Sjögren's syndrome

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Abstract

Recent studies have suggested that vitamin D_3 may have an immunoregulatory role in vitro. The vitamin D_3 metabolism in 35 patients with primary Sjögren's syndrome was investigated by measuring blood concentrations of 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) and 25-hydroxyvitamin D_3 (25-OHD_3), as well as phenotypes and blood concentrations of Gc globulin, the main vitamin D_3 binding protein in the blood. 25-OHD_3 concentrations were diminished, but those of 1α,25-$(OH)_2D_3$ were normal. There was no significant difference between the distribution of Gc phenotypes in the patients with primary Sjögren's syndrome and normal controls. Likewise, blood concentrations of Gc globulin corresponded to normal values. Among patients with increased concentrations of IgM rheumatoid factor there was a significant negative correlation between the serum titres of IgM rheumatoid factor and 25-OHD_3 concentrations.

Cytoplasmic receptors for 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) have been identified in normal human monocytes as well as in activated, but not resting, human B and T lymphocytes.[1 2] Moreover, pulmonary alveolar macrophages from patients with sarcoidosis are capable of 1α-hydroxylation, which converts 25-OHD_3 to the biologically most active form—1α,25-$(OH)_2D_3$.[3] Furthermore, interferon gamma or lipopolysaccharide can induce normal human monocytes and macrophages to exhibit 1α-hydroxylase activity.[4]

In vitro studies suggest that 1α,25-$(OH)_2D_3$ has an immunoregulatory role. The hormone induces differentiation of monocytes[5] and it blocks the production of interleukin-2[6] and immunoglobulin,[7] possibly by inhibiting the lymphocyte activating interleukin-1.[8]

Primary Sjögren's syndrome is a chronic autoimmune disease characterised by lymphoid infiltration of exocrine glands, especially lachrymal and salivary glands.[9] Polyclonal B cell activation with hypergammaglobulinaemia[10] and the presence of a broad range of autoantibodies, including IgM and IgA rheumatoid factors, as well as antinuclear antibodies, are characteristic of the syndrome.

The aim of this study was to investigate whether disturbed vitamin D_3 metabolism accompanies primary Sjögren's syndrome, by examining blood concentrations of vitamin D_3 and phenotypes of Gc globulin, the main vitamin D_3 binding protein in the blood.

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Methods

Thirty five consecutive female patients with primary Sjögren's syndrome were studied. Their ages ranged from 27 to 69 years, mean 51 years. All patients fulfilled the Copenhagen diagnostic criteria for primary Sjögren's syndrome[11]—that is, keratoconjunctivitis sicca evaluated by the Schirmer-1 test, break-up time, and van Bijsterveld score, and xerostomia determined by unstimulated sialometry, salivary gland scintigraphy, and lower lip biopsy. The patients were not treated with immunosuppressive drugs other than non-steroidal anti-inflammatory drugs (n=4). The most common extraglandular manifestations were as follows: arthralgia/synovitis (n=18, 51%), myalgia/myositis (n=7, 20%), Raynaud's phenomenon (n=8, 23%) and pulmonary signs (n=7, 20%). Only a limited number of the patients showed other extraglandular manifestations such as purpura, interstitial nephritis, cutaneous vasculitis, intermittent non-infectious fever, and lymphoproliferative disorders. Serum samples were stored at −20°C until assay.

Serum 25-OHD_3 was examined by a radioassay using rachitic rat kidney cytosol as a binding protein. The normal mean (SD) was 28·2 (12·4) ng/ml (taken from healthy volunteers of both sexes, aged 18 to 60 years, from whom blood samples were obtained throughout the year; the mean therefore represents a sex, age, and seasonal average).[12] Serum 1α,25-$(OH)_2D_3$ was measured by a modification of a method described previously.[13] Calf thymus receptors were used as a source of binding protein. Normal mean (SD) was 33·1 (15·3) pg/ml (healthy volunteers of both sexes aged 18–86 years). In these assays vitamin D_2 is measured in addition to vitamin D_3.

Ionised calcium was measured, as described previously.[14] The normal range (pH=7·4) is 1·15–1·35 mmol/l.

Gc typing was performed by isoelectric focusing, followed by specific immunofixation on cellulose acetate.[15] Gc levels were determined by rocket immunoelectrophoresis using purified human Gc globulin (Sigma Chemical Company, St Louis, Missouri, USA) as standard and polyclonal rabbit anti-Gc-antibody (Dakopatts, Denmark) (normal range 0·4–0·7 mg/ml).[16]

The concentrations of IgA and IgM rheumatoid factors were determined by an enzyme linked immunosorbent assay (ELISA), as described previously.[17] Concentrations of IgM rheumatoid factor were determined by relating the content in the samples to an internal laboratory standard with a known concentration

of IgM rheumatoid factor expressed in international units (IU) (normal value <8 IU/ml). IgA rheumatoid factor concentrations were related to an internal laboratory standard serum and the content expressed in arbitrary units (AU) (normal value <25 AU/ml).

Titres of circulating antinuclear antibodies of the IgG class (determined by an indirect fluorescence technique) and concentrations of plasma immunoglobulins were recorded at the same time.

The Pearson correlation coefficient and the χ^2 of test was used. A level of 5% was regarded as significant.

Results

The blood concentrations of $1\alpha,25\text{-}(OH)_2D_3$ in patients with Sjögren's syndrome (median 26·0, Q_1 19·0, Q_3 42·0 pg/ml) were normal (mean (SD) 33·1 (15·3) pg/ml). In contrast, the concentrations of $25\text{-}OHD_3$ were reduced (median 9·0, Q_1 5·0, Q_3 16·0 ng/ml), (normal mean: 28·2 (12·4) ng/ml). Undetectable concentrations of $25\text{-}OHD_3$ were found in nine patients, and six of these had serum $1\alpha,25\text{-}(OH)_2D_3$ concentrations lower than 15 pg/ml (figure).

There was no significant difference between the distribution of Gc phenotypes in the patients with primary Sjögren's syndrome and in 1674 controls ($\chi^2=1\cdot65$) (table). The serum levels of Gc globulin (median 0·6 mg/ml, Q_1 0·55, Q_3 0·65) corresponded to normal values (0·4–0·7 mg/ml serum).

Increased concentrations of IgM rheumatoid factor were found in 25 (72%) patients, with a median concentration of 118 IU/ml (Q_1 41, Q_3 189). Increased concentrations of IgA rheumatoid factor were found in 21 (60%) of the patients, with a median concentration of 92 AU/ml (Q_1 55, Q_3 105). Among patients with increased concentrations of IgM rheumatoid factor there was a significant negative correlation ($r=-0\cdot42$, $p<0\cdot05$) between the titres of IgM rheumatoid factor and the concentration of $25\text{-}OHD_3$ but not those of $1\alpha,25\text{-}(OH)_2D_3$. This was, however, not found within the group of IgA rheumatoid factor positive patients.

Twenty four (69%) of the patients exhibited antinuclear antibodies. The concentration of $1\alpha,25\text{-}(OH)_2D_3$ and $25\text{-}OHD_3$ within this group did not differ significantly from the group of patients as a whole.

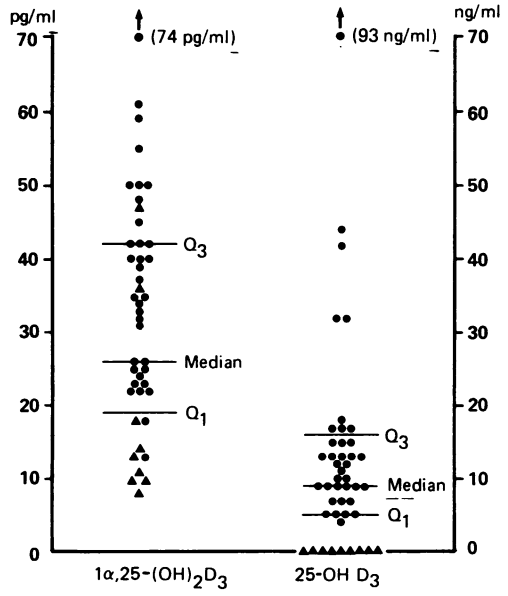
Sixteen (46%) had hypergammaglobulinaemia (IgG, IgA, IgM). The median IgG level was 14·9 g/l (Q_1 12·7, Q_3 25·2 g/l). Neither immunoglobulin levels nor the presence of extraglandular manifestations correlated with the vitamin D_3 concentrations.

Ionised calcium concentration in 12 patients from whom additional serum samples were available (median 1·19 mmol/l, Q_1 1·14, Q_3 1·23) was normal (1·15–1·35 mmol/l).

Gc phenotype distribution in 35 patients with primary Sjögren's syndrome

Number	*Gc phenotypes*					
	1F	*1S*	*2*	*1F–1S*	*2–1F*	*2–1S*
Observed	0	13	3	8	3	8
Expected*	0·9	11·4	2·5	6·4	3·0	10·8

*Calculated from Gc prevalence determined in 1674 unrelated Danish adults.



Distribution of $1\alpha,25$-dihydroxyvitamin D_3 ($1\alpha,25\text{-}(OH)_2D_3$) and 25-hydroxyvitamin D_3 ($25\text{-}OHD_3$) in 35 patients with primary Sjögren's syndrome. ($1\alpha,25\text{-}(OH)_2D_3$: normal mean (SD) is 33·1 (15·3) pg/ml; $25\text{-}OHD_3$: normal mean (SD) is 28·2 (12·4) ng/ml. ▲=patients with undetectable concentrations of $25\text{-}OHD_3$.

Discussion

Primary Sjögren's syndrome was chosen for two reasons to examine the hypothesis that vitamin D metabolism is changed in patients with systemic autoimmune diseases. Firstly, polyclonal B cell activation is a pronounced and convenient marker of disturbed immune regulation in this disease. Secondly, patients with primary Sjögren's syndrome are normally not treated with immunosuppressive drugs that may affect metabolic rate. To our knowledge, this study is the first investigation of vitamin D metabolism in patients with primary Sjögren's syndrome. We found that the serum concentrations of $25\text{-}OHD_3$ were significantly below those of normal subjects in most patients with primary Sjögren's syndrome. In contrast, the median concentration of $1\alpha,25\text{-}(OH)_2D_3$ was normal. Serum immunoglobulins and immune complexes do not interfere with the vitamin D_3 assay.

It is questionable whether the changed vitamin D metabolism affects immune reactivities and contributes to the polyclonal B cell activation that is characteristic of these patients. Because $1\alpha,25\text{-}(OH)_2D_3$ is, immunologically, the most active vitamin D metabolite in vitro, it is conceivable that an abnormal concentration of this compound might result in changed immune function.[1 7 8] Low concentrations (≤18 pg/ml), however, were only found in eight of the 35 patients and did not correlate with the titres of immunoglobulin or autoantibodies. $25\text{-}OHD_3$ has been found to be about a thousand times less active than $1\alpha,25\text{-}(OH)_2D_3$ in vitro,[6 8 18] and it has a higher affinity for Gc globulin.[19] As

the normal blood concentrations of 25-OHD_3 are about a thousand times higher than those of 1α,25-$(OH)_2D_3$ the observed low concentrations of 25-OHD_3 might have affected immune reactivity in these patients. It is interesting, therefore, that a highly significant correlation between the concentration of circulating 25-OHD_3 and IgG titres has been reported.[20] This was not found in our study, but the negative correlation between 25-OHD_3 concentrations and IgM rheumatoid factor suggests that vitamin D_3 metabolism may be related to immunopathological processes in primary Sjögren's syndrome.

What might be the cause of the observed low 25-OHD_3 concentrations? One possibility might be an impaired ability of Gc globulin to bind D_3 vitamins. Thus certain phenotypes of Gc globulin have been associated with other immunoinflammatory diseases, such as rheumatoid arthritis,[21] multiple sclerosis,[22] and liver disease.[23] Eales and colleagues also recently reported an association between different Gc alleles and the susceptibility to, and the clinical manifestation of, human immunodeficiency virus (HIV) infection.[24] Subsequent studies, were, however, unable to confirm this finding.[25] In our patients with primary Sjögren's syndrome the distribution of Gc phenotypes did not differ significantly from that in the normal population. Moreover, the serum concentrations of Gc globulin were normal. Therefore, changed Gc globulin binding of vitamin D_3 is unlikely to account for the low 25-OHD_3 concentrations found in patients with primary Sjögren's syndrome.

Low concentrations of vitamin D_3 may also result from severely impaired liver function[26] but our patients had no signs of liver disease which might have explained the concentrations of 25-OHD_3 we found.

As the primary aim of this investigation was to study the vitamin D_3 metabolism, and because the concentrations of ionised calcium were normal, the parathyroid hormone and calcitonin metabolism were not likely to have been disturbed and were therefore not studied.

Finally, polyclonal B cell activation may itself have contributed to the low concentrations of 25-OHD_3, because 25-OHD_3 is metabolised in activated monocytes and consumed by activated lymphocytes through binding to vitamin D receptors of these cells.[1 7]

In conclusion, we found severely diminished blood concentrations of 25-OHD_3 in patients with primary Sjögren's syndrome. It is unclear whether the changed vitamin D_3 metabolism is related to the immunopathology of this disease or is merely an epiphenomenon.

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